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14. ABSTRACT Widely recognized as the gold standard for biological recognition, antibodies have been used to develop bioscavengers, enzyme-like catalytic therapies, and highly specific diagnostic clinical tests. The discovery of camelid antibodies in the 1990s has extended the useful range of antibodies into pH extremes, high temperature, and high salt conditions due to increased stability. However, determining the binding properties of these molecules prior to use under these conditions has not been widely employed because the most precise biophysical methods, surface plasmon resonance and isothermal titration calorimetry, are not compatible with highly complex matrices. Here we demonstrate the use of microscale thermophoresis as a rapid, simple method to detect binding of a camelid antibody in the presence of jet fuel/water mixes; a complex, harsh environment. This work provides key data to quantifying binding for the development of a VHH-based sensor for detection of microbial growth in fuel.

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MEASURING SINGLE-DOMAIN ANTIBODY INTERACTIONS WITH EPITOPES IN JET FUEL USING MICROSCALE THERMOPHORESIS

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Widely recognized as the gold standard for biological recognition, antibodies have been used to develop bioscavengers, enzyme-like catalytic therapies, and highly specific diagnostic clinical tests. The discovery of camelid antibodies in the 1990s has extended the useful range of antibodies into pH extremes, high temperature, and high salt conditions due to increased stability. However, determining the binding properties of these molecules prior to use under these conditions has not been widely employed because the most precise biophysical methods, surface plasmon resonance, and isothermal titration calorimetry, are not compatible with highly complex matrices. Herein, we demonstrate the use of microscale thermophoresis as a rapid, simple method to detect binding of a single-domain antibody in the presence of jet fuellwater mixes; a complex, harsh environment. This work provides key data to quantifying binding for the development of a sensor for detection of microbial growth in fuel.

Keywords: Microscale thermophoresis; Nanobody; Single domain antibody; VHH-domain

ABBREVIATIONS

LcrV, low-Ca^{2+} response V-antigen; sdAb, single-domain antibody; MST, microscale thermophoresis; SPR, surface plasmon resonance

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INTRODUCTION

Microbial contaminants in various environments including fuel tanks, water storage systems, exterior surfaces of ships, medical facilities, and office spaces are commonly associated with decreased material performance or increased risk of human illness. Early detection of contamination in these locations could result in assurance of area performance and health conditions with validation of clean equipment and consumables. Antibodies are widely recognized as the ideal solution for situations in which high affinity, selectivity, and sensitivity are required for sensing and detection. Recent uses include immobilized, label-free detection of a cardiac cell death marker troponin I (Zhang et al. 2014) and the explosive trinitrotoluene (Charles et al. 2014), as well as use in lateral-flow and cellulose paper sensors for biological targets (Zhao et al. 2014; Zhu et al. 2014). However, the wide use of antibodies for sensing in unique and harsh environments is precluded because they are expensive to produce and unstable in all but the most optimized conditions. Aptamers have provided a popular alternative because they are highly stable, simple to synthesize, have low immunogenicity and can identify targets against which raising quality monoclonal antibodies has proven difficult (Song, Lee, and Ban 2012). Aptamers, however, do include their own challenges as their isolation through selection processes are not always successful and the high negative charge of the polymer backbone presents difficulties in some recognition events. An option combining the best of both of these recognition elements required in optimizing selective biological binding is the use of a single-domain antibody (sdAb). These single-chain antibodies contain the same recognition properties of standard antibodies but are much smaller with a stability similar to aptamers (Trilling et al. 2011; Perez et al. 2001).

Jet fuel is a highly complex mixture containing volatile and semi-volatile organic compounds. As with many other diverse environments, a number of microbes, such as *Sphingomonas* and *Pseudomonas*, have evolved to survive in aqueous pockets within oils and fuels (Baraniecki, Aislabie, and Foght 2002; Itah et al. 2009). The biofilms produced when such microbes reach a critical quorum threshold are resistant to traditional cleaning solutions and techniques, leading to aggressive processes of preventative cleaning. Such approaches are time-consuming, expensive and frequently a significant toxicity risk to maintenance personnel. Recent research has detailed adverse toxic effects after exposure to jet fuel including hearing loss (Fechter et al. 2012), mental impairment (Maule et al. 2013), and DNA damage (Krieg et al. 2012). In addition, allowing uncontrolled microbial growth in the fuel may lead to disruptive issues including filter plugging, injector fouling, tank topcoat peeling, biocorrosion, and fuel degradation (Passman 2012).

Herein, we used nanobodies developed to the yersinia excreted protein LcrV (low-Ca^{2+} response V antigen) as a proof-of-concept for bacterial testing in sump water/jet fuel mix to characterize the binding interactions in such complex environments. Utilizing the gold-standard biophysical technique, surface plasmon resonance (SPR), dissociation constants (K_ds) were determined for the proteins in buffered solution. Subsequently, microscale thermophoresis (MST) was utilized to confirm the observations and to compare the values of an immobilized versus labeled target, respectively. Finally, to demonstrate the robustness of the sdAb-based recognition, MST was used to test binding in jet fuels.

EXPERIMENTAL

Recombinant LcrV was expressed and purified from *E. coli* using affinity chromatography on an AKTAExplorer (GE Healthcare). The sdAbs were developed by Dr. Serge Muyldermans (Flanders Institute for Biotechnology). Data analysis for both MST and SPR was performed using Prism 5 (GraphPad Software) and errors presented are standard deviations of triplicate measurements. MST was performed using LcrV fluorescently-labeled with DyLight 650 (Pierce) on a Monolith NT.115 (NanoTemper Technologies) in standard treated capillaries. SPR was performed on a Biacore T200 (GE Healthcare) by immobilizing LcrV on a Series S Sensor Chip CM5 (GE Healthcare) using an Amine Coupling Kit (GE Healthcare). Detailed procedures are provided as supplementary information.

RESULTS AND DISCUSSION

Initial characterization of the binding affinities for the sdAb antibodies (L1 and L3) was performed in aqueous buffer (Figure 1). As an off-rate was not observed in either the L1 or L3 sensorgrams, K_ds was calculated for both interactions using the equilibrium method (Dell'Orco, Mueller, and Koch 2010). As expected for sdAb antibodies, the K_ds were all in the low nanomolar range (9.4 and 36.7, respectively).

Due to the delicate nature of the instrumentation in the SPR, MST was used to test binding in a mixture of jet fuel and sump water. To first confirm that MST would be a reliable method for comparison, we performed sdAb titrations in aqueous buffer (Figure 2). As expected from previous published works, the K_ds determined

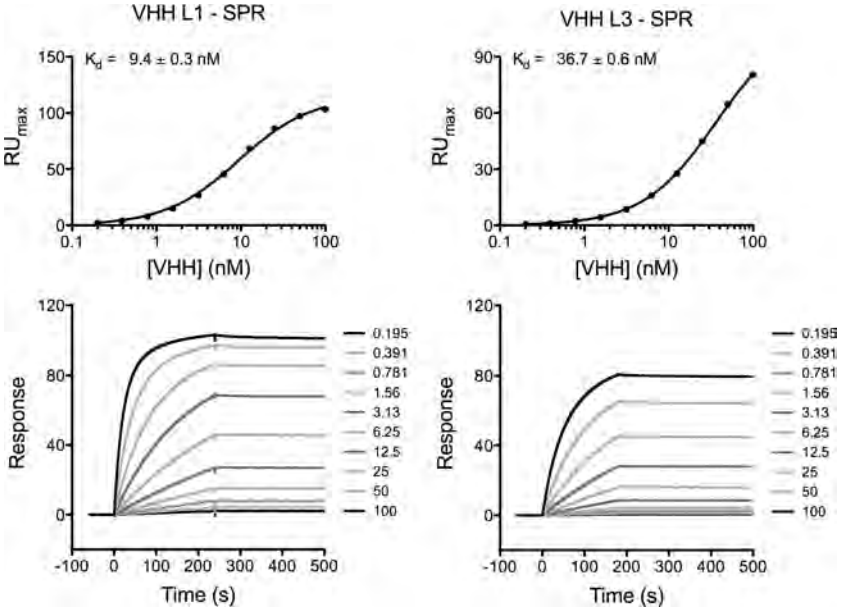

Figure 1. Surface plasmon resonance characterization of immobilized LcrV binding by single-domain antibodies L1 (left) and L3 (right). The dissociation constants were calculated using the RU_{max} after equilibrium was established (top) due to lack of kinetic off-rates in the sensorgrams (bottom).

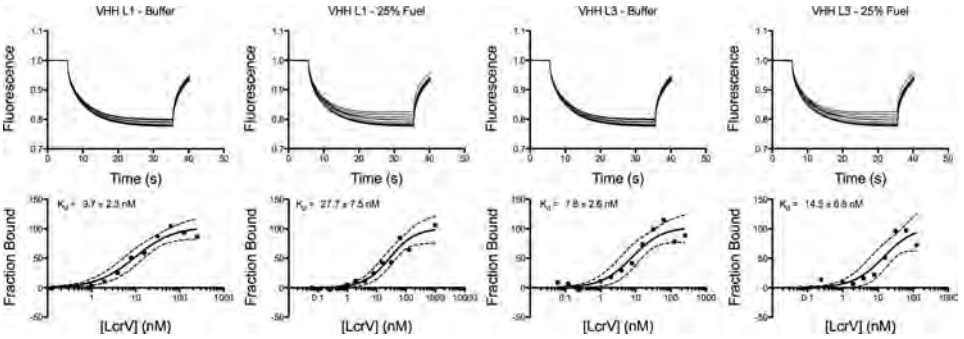

Figure 2. LcrV binding to single-domain antibodies L1 and L3 in buffer and a jet fuel-sump water mixture. Thermophoretic timetraces (top) demonstrate neither aggregation nor adsorption to the capillary walls. Individual binding curves (bottom) clearly show consistency of data with low noise. Fraction bound shown with 99% confidence bands.

from MST were consistent with SPR with similar discrepancies to the literature (Ramakrishnan et al. 2012).

Interestingly, the jet fuel-sump water mixture resulted in minimal impact on K_ds for the two nanobodies (Figure 2). The variation in MST-based binding affinities was not significant (9.7 vs. 27.7 nM for L1, and 7.8 vs. 14.5 nM for L3). When considering the deviations observed between K_ds determined by SPR and MST for L1 and L3, these changes are negligible.

MST provides a valuable tool for real-world biophysical characterizations. With its disposable glass capillaries, harsh samples that require laborious cleanup for use in standard methods can be used directly. This feature highlights the broad applicability of the tool. It can be envisioned that polymer-coated nanomaterials could be used to capture contaminants in even more extreme conditions, such as in detergents (i.e., face wash). The list of as-yet untested sample matrices is vast and is limited really only by the recognition elements involved. We have demonstrated the use of MST to characterize contaminants in jet fuel, yet one can imagine testing in bleach, sea water, alkaline vents, and many others.

CONCLUSIONS

We have demonstrated that nanobodies are capable of recognizing LcrV with high affinity in organic/aqueous mixtures of jet fuel/sump water. Recent advances in biophysical and bioanalytical methods were employed to demonstrate that two of our camelid antibodies maintain the ability to recognize their target in a mixture of jet fuel and water. This work presented suggests that these antibodies and camelid sdAb-domains in general, may have the robustness in affinity to be applied in real-world fuel tank biosensors for early detection of biocontamination.

SUPPLEMENTAL MATERIAL

Supplemental data for this article can be accessed on the publisher's website at http://dx.doi.org/10.1080/00032719.2014.947535.

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